

## Symbiosis of the endangered *Lupinus mariae-josephae* lupin species: Successful “in situ” propagation with rhizobial inoculation

Durán D<sup>1</sup>, Navarro A<sup>2</sup>, Rey L<sup>1</sup>, Imperial J<sup>1,3</sup>, Ruiz-Argüeso T<sup>1\*</sup>.

<sup>1</sup>Centro de Biotecnología y Genómica de Plantas (CBGP) and Departamento de Biotecnología (ETSIA), Universidad Politécnica de Madrid, Campus de Montegancedo, Pozuelo de Alarcón (Madrid), Spain, <sup>2</sup>Centro para la Investigación y Experimentación Forestal (CIEF), Generalitat Valenciana, Quart de Poblet, Valencia, Spain, <sup>3</sup>CSIC, Madrid, Spain

*Lupinus mariae-josephae* (Lmj) is a lupine endemism that is only found in soils from a small area in Valencia region, in Eastern Spain. This lupine thrives in alkaline soils with high pH, a unique habitat for lupines. In these soils, Lmj grows in just a few defined patches, and previous conservation efforts directed towards controlled plant reproduction have been unsuccessful. A legislative decree (70/2009, page 20156 Anex I) published in the el 'Diario Oficial de la Comunitat Valenciana' shows Lmj in a category corresponding, in the latest version of the Red List of IUCN (IUCN, 2012) (International Union for Conservation of Nature and Nature Resources), to an “Endangered” legume species not extinct in the wild. Most current IUCN criteria used to define rare, small-range legume species, are based on history of reproductive traits such as number of pods and seeds. We have previously shown that Lmj plants establish a specific root nodule symbiosis with bradyrhizobia present in those soils, and we reasoned that the paucity of these bacteria in soils might contribute to the lack of success in reproducing plants for conservation purposes. Greenhouse experiments using Lmj trap-plants showed an absence, or very low concentration, of Lmj-nodulating bacteria in “terra rossa” soils of Valencia outside of Lmj plant patches. No Lmj endosymbiotic bacteria were found in “terra rossa” or alkaline red soils outside the Valencia Lmj endemism region in the Iberian Peninsula or Balearic Islands. Among the rhizobia able to establish an efficient symbiosis with *L. mariae-josephae* plants, two *Bradyrhizobium* sp. strains, LmjC and LmjM3, were selected as inocula for seed coating. Two planting experiments were carried out in consecutive years under natural conditions in areas with edapho-climatic characteristics identical to those sustaining natural Lmj populations, and successful reproduction of the plant was achieved. Interestingly, the successful reproductive cycle was absolutely dependent on seedling inoculation with effective bradyrhizobia, and optimal performance was observed in plants inoculated with LmjC, a strain that had previously shown the most efficient behavior under controlled conditions. These results define conditions for *L. mariae-josephae* conservation and for extension to alkaline-limed soil habitats, where no other known lupine can thrive. Broadly speaking, the work singularly identified the rhizobial symbiosis as a factor affecting the conservation of legumes and often being exceedingly vulnerable to threats. Our results also indicate that seed inoculation with N<sub>2</sub>-fixing, efficient Rhizobium strains is a strategy to consider in the conservation of endangered legume species

### References

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